

Amendments to the Specification

Amend the paragraph beginning on page 19, line 4 as follows.

A DNA partial duplex consisting of an 18-mer primer 5' —5
CAACGTCCGAGCAGTACA (SEQ ID NO:1) and a 40-mer template 5' —
AAGCTCCTTAGTCTGTCAATGTACTGCTCGGACGTTGCGA (SEQ ID NO:2) (Fig. 3A)
was prepared by annealing PAGE-purified oligonucleotides. DNA duplex (2 μ M) was
incubated in 20 μ l polymerase reaction mixture (6.7 mM tris-HCl pH 8.8, 6.6 mM MgCl₂, 1
mM DTT, 16.8 mM (NH₄)₂SO₄, 200 μ M dNTPs and 0.25 U/ μ l Klenow fragment or T4 DNA
polymerase) for 20 min at room temperature. Reactions were stopped with an equal volume of
gel loading buffer (98% formamide, 10 mM EDTA pH 8.0, 0.025% bromophenol blue, 0.025%
xylene cyapole FF), heated at 95°C for 2 min and subjected to denaturing PAGE (see Fig.3B).
The substrate properties of Fc-dUTP were tested in DNA polymerase-catalysed primer
extension assays using the model DNA duplex shown in Fig. 3A. The sequence of the template
allows the progress of primer extensions to be controlled by omitting some dNTPs from the
reaction mixture. The results of incubating primer-template with E. coli DNA polymerase I
Klenow fragment or T4 DNA polymerase are shown in Fig. 3B. Addition of unlabelled dTTP to
the reaction mix results in extension of the 18-mer primer (lane 1) by 2 nucleotides (lanes 2 and
8). The product heterogeneity displayed by T4 DNA polymerase (lane 8) is caused by its
stronger 3'-5' exonuclease activity, which is also evidence in lanes 9-11. When Fc-dUTP
replaces dTTP, both DNA polymerases incorporate two consecutive Fc-dUMP residues into the
3'-end of the primer (lanes 3 and 9). Because the incorporated pFc-dU residue has a molecular
weight almost twice that of the pT residue (574 vs. 321 Da) and the bulky adduct also alters the
hydrodynamic properties of the chain, the mobility of the Fc-dUTP-extended primer is
significantly lower than that of its natural counterpart. There is an indication that a small
fraction of the primer is not extended by Klenow fragment (lane 3), but this behaviour is not
consistent across the gel.

Amend the paragraph beginning on page 20, line 15 as follows.

A segment of the T4 DNA ligase gene (positions 1001 to 1988) was use as a model sequence for amplification in the poresence of a ferrocene-labelled TTP analogue. The gene was cloned into plasmid pKLO1. The 25-mer 5'-GCT GAT GGA GCT COG TGT TTT GCT T-3' (SEQ ID NO:3) was used as a forward primer, and 31-mer 5' - TAT ATA AGC TTC ATA GAC CAG TTA CCT CAT G-3' (SEQ ID NO:4) was used as a reverse primer. The use of 20 these primers allows formation of a 998 nt long amplicon. The reaction mixtures (20 μ L each) contained 6.7mM tris-HCl (pH 8.8), 1.66 mM $(\text{NH}_4)_2\text{SO}_4$, 0.045% Triton X-100, 0.02 mg/mL gelatin, 2.5 mM MgCl_2 , 0.2 μ M each primer, 20 ug/mL pKLO1 plasmid, 0.2 mM dNTPs, and 0.1 U/uL *Tth* polymerase (exo). In some reaction mixtures, TTP was partially or fully substituted with Fc-dUTP in such a way that the total concentration of TTP and Fc-dUTP was still 0.2 mM. Conditions of PCR were as follows: 2 mm at 95°C, and then 22 cycles at 94°C for 30 see, 50°C for 30 see, 50°C for 1 mm, and 70°C for 10 min. After amplification, 4uL of gel loading buffer (30% glycerol, 0.25% bromphenol blue and 0.25% xylene cyanole FF) was added, and samples were analysed on 1% agarose gel.

Amend the paragraph beginning on page 22, line 1 as follows.

4 μ M duplex DNA (40-mer template 5' — AAGCTCCTTAGTCTGTCAATGTACTGCT CGGACGTTGCTA-3' (SEQ ID NO:5) and 18-mer primer 5' — CAACGTCCGAGCAGTACA-3' (SEQ ID NO:6)) was incubated in 240 μ L of reaction mixture consisting of 6.7mM tris-HCl (pH 8.8), 6.6 mM MgCl_2 , 1 mM DTT, 16.8 mM $(\text{NH}_4)_2\text{SO}_4$, 200 μ M dNTPs (except TTP), 200 μ M Fc-dUTP, and 0.25 U/ μ L of Kienow fragment for 20 mm at room temperature. Low molecular weight components were separated on Bio-Spin 30 chromatography column (Bio-Rad). The eluate was extracted with equal volumes of phenollchloroform (1:1) and chlorophorm. DNA was precipitated by addition of 10 volumes of 2% LiClO_4 in acetone and centrifugation (12000g, 15 min). Precipitate was dried *in vacuo*, redissolved in 200 μ L of HPLC buffer (50 mM LiClO_4 /2.5% acetonitrile in water), and the DNA concentration was determined spectrophotometrically by absorption at 260 nm. Different amounts of sample were loaded onto the analytical reverse-phase column (Vydac, Protein & Peptide C18, 250 x 4mm) and analysed by isocratic elution with optical (260 nm) and electrochemical ($E = 0.7\text{V}$) detections (flow rate — 1 mL/min). After being extended in the presence of all 4 dNTPs including Fc-dUTP instead of TTP, the model DNA duplex would

contain five Fc-dUTP residues. We used this ferrocene-labelled duplex for electrochemical detection in the course of RP HPLC. The HPLC system was equipped with both optical and electrochemical detectors as described in Materials and Methods. Different quantities of DNA duplex were injected on the reverse phase column and eluted in isocratic mode by 50 mM LiClO₄ / 2.5% acetonitrile in water. The eluate was monitored optically at 260 nm and electrochemically at 0.7 V. In our conditions, the retention time for DNA duplex was 17.5 min. Only picomolar quantities of DNA were reliably detected with UV/VIS photo array detector, while electrochemical detection allowed to register femtomolar amounts of the duplex (Fig.4).

Add the sequence listing, enclosed herewith, to the end of the specification.